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THE TITRATION OF DIPHTHERIA TOXIN IN UNILATERALLY NEPHRECTOMIZED GUINEA-PIGS *

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Experimental observations on the effect of variation in the elimination of bacterial toxins in an animal on its susceptibility to their toxic effect are entirely lacking. The occurrence of distinctive lesions in the kidneys of animals succumbing to diphtheria toxin (Welch and Flexner,[1] Frothingham,[2] Bailey[3]), as well as the frequent occurrence of nephritis in diphtheria patients indicates the importance of the kidney in the elimination of this poison and that variation in renal functional activity may materially affect susceptibility to diphtheria toxin. The object of the present study was to determine the effect of decreased kidney substance, produced by nephrectomy, on the fatal dose of diphtheria toxin.

The left kidney was removed through a half-inch incision in the posterior and upper left lumbar region. Animals with external wound infections following nephrectomy were discarded. The guinea-pigs weighed between 240 and 300 gm. The control animals were normal pigs, splenectomized pigs, and animals in which a piece of omentum had been removed. In all of the experiments the same toxin was used. It had a standard L + dose of 0.2 c.c. and was obtained from the U. S. Public Health Service at Washington, D. C. The dosage was graduated in the same manner as is customary in the titration of an unknown diphtheria toxin. The urine of most of the animals was tested for albumin shortly before injection of the toxin. The animals were carefully observed for toxic symptoms and examined carefully after death for characteristic hemorrhagic edema and induration about the site of injection, hemorrhagic or congested suprarenals, and focal necrosis of the liver. Owing to the well known susceptibility of guinea-pigs to intercurrent infections and sudden fatal epidemics, pneumonia, pleurisy, ascites, and peritonitis were not regarded as evidence of death due to diphtheria toxin without coincident lesions more characteristic of diphtheria intoxication.

In the 1st series of experiments the effect of the nephrectomy was determined on the M. L. D. The results showed a marked diminution

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[1] Bull. Johns Hopkins Hosp., 1891, 2, p. 107.
[2] Jour. Med. Research, 1914, 30, p. 365.
[3] Jour. Exper. Med., 1917, 25, p. 109.

TABLE 1

TITRATION OF DIPHTHERIA TOXIN

	Determination of L+ Dose					
		Results				
	Dose, C.c.	Death	Local Reaction	Supra-renal Congestion	Liver Necrosis	Miscellaneous
Unilaterally nephrectomized guinea-pigs	0.2	48 hr.	±	±	—	—
	0.18	60 hr.	+	—	—	
	0.16	72 hr.	+	+	—	
	0.14	......	..	..	..	Recovery
	0.12	......	..	..	..	Recovery
Normal control guinea-pigs	0.2	90 hr.	+	+	—	
	0.18	......	..	..	..	Recovery
	0.16	......	..	..	..	Recovery
	0.14	......	..	..	..	No symptoms
	0.12	60 hr.	—	—	—	Pneumonia
Splenectomized guinea-pigs	0.2	7 days	—	—	—	
	0.18	......	..	..	..	Recovery
	0.16	......	..	..	..	Recovery
	0.14	......	..	..	..	No symptoms
	0.12	......	..	..	..	No symptoms

TABLE 2

TITRATION OF DIPHTHERIA TOXIN WITH ANTITOXIN

Subject	Number	Weight, Gm.	Weight of Left Kidney, Gm.	Post-Operation	Albumin in Urine
Unilaterally nephrectomized guinea-pigs	162	276	1	24 hr.	—
	159	266	1.34	48 hr.	—
	86	260	1.38	96 hr.	—
	68	244	1.25	9 days	—
	64	268	1.35	14 days	—
	123	220	1.45	21 days	—
	120	325	1.8	30 days	—
Control guinea-pigs*	163	269	....	24 hr.	..
	161	266	....	48 hr.	..
	67	215	....	96 hr.	..
	76	235	....	7 days	..
	54	230	....	14 days	..

TABLE 1—*Continued*

Titration of Diphtheria Toxin

Determination of M. L. D.*

Dose, C.c.	Results				
	Death	Local Reaction	Suprarenal Congestion	Liver Necrosis	Miscellaneous
0.01	48 hr.	±	±	—	—
0.008	72 hr.	+	+	—	—
0.006	76 hr.	+	+	—	—
0.005	90 hr.	±	±	—	—
0.004	6 days	++	±	++	—
0.003	90 hr.	—	—	—	Peritonitis local infection
0.01	6 days	—	±	—	—
0.008	76 hr.	+	+	—	—
0.006	90 hr.	+	+	+	—
0.005	......	..	..	..	Recovery
0.004	5 days	+	+	—	—
0.003	......	..	..	..	Recovery

* Control animals were subjected to laparotomy with removal of a piece of omentum.

TABLE 2—*Continued*

Titration of Diphtheria Toxin with Antitoxin

Dose, C.c.	Results					
	Death	Local Reaction	Suprarenal Congestion	Liver Necrosis	Weight Right Kidney, Gm.	Miscellaneous
0.16	54 hr.	+	+	—	1.4	
0.16	52 hr.	+	+	+	1.8	
0.16	60 hr.	+	+	—	1.8	
0.16	73 hr.	+	+	—	1.93	
0.16	76 hr.	+	+	—	2.2	
0.16	66 hr.	+	+	+	1.73	
0.16	......	..	..	..	...	Recovery
0.16	......	..	..	..	...	Symptoms with recovery
0.16	......	..	..	..	...	Symptoms with recovery
0.16	86 hr.	+	+			
0.16	......	..	..	..	...	No symptoms
0.16	60 hr.	+	+	..	...	Local infection at tail of pancreas

* Laparotomy with removal of some of omental fat.

in the resistance of the nephrectomized animals as compared with normal pigs. When, however, the operative factor with its coincident traumatism and shock was minimized by the use of controls of splenectomized pigs or pigs in which a piece of omentum had been removed, the difference in the resistance was not so great, though still distinctly in favor of the control animal. This is illustrated in Table 1, which gives a summary of the results of 1 of these experiments in which all of the nephrectomized animals succumbed while 2 of the control animals survived. In this experiment it is also of interest that the nephrectomized animal that received the smallest dose of toxin had a trace of albumin in the urine and died before one receiving a larger dose. In another experiment, 1 of the control animals, the urine of which showed a trace of albumin, also showed a decided susceptibility to the toxin.

The 2nd series of experiments was made to determine the effect of nephrectomy upon the L+ dose. When compared with the normal control animals the increased susceptibility of the nephrectomized animals was very striking. With the elimination of the operating factor by the use of splenectomized animals as controls, the resistance of the nephrectomized pigs was also considerably diminished (Table 1).

A final experiment was made to determine the effect of the postoperative period and of the compensatory hypertrophy of the right kidney on the L+ dose. The data of this experiment are summarized in Table 2. The number of guinea-pigs used includes a series operated on at different intervals, but receiving a constant dose of diphtheria toxin. The control animals were operated on approximately the same date, with removal of a section of omentum. In the 1st place, they show conclusively the increased susceptibility of the nephrectomized animals, all but 1 dying while only 2 of the control animals died, and one of these, No. 54, had an operative infection which contributed to its susceptibility, especially since this was the animal with the longest postoperative period and one that otherwise would be expected to survive all others. In the 2nd place, the data point to the relative unimportance of the time elapsing after the operation (under 3 weeks) on the animal's resistance. Furthermore, the degree of compensatory hypertrophy (ascertained by weight) of the remaining kidney does not bear any consistent relation to the resistance to the toxin. For example, Animals No. 68 and No. 64 showed a compensatory hypertrophy of 39% and 63%, respectively, with no appreciable difference in their resistance, this in spite of the fact that No. 64 had 5 more days' rest after the operation. In other words, increase of kidney substance alone does not cause corresponding increase of resistance. The recovery of No. 120 suggests, however, that the functional activity of the kidney falls behind its anatomic hypertrophy, but will in time fully compensate. There is also considerable variation in the amount of compensatory hypertrophy that occurs in different animals and, in every instance, it falls considerably, usually less than 50%, short of the combined weights of both kidneys before operation. The hypertrophy occurs mainly within the first few days. It is also evident from the behavior of the control animals that guinea-pigs present considerable individual variation, a fact that would make confirmation of such experiments as these very desirable.

The effect of nephrectomy on the M. L. D. is to lower it. This is probably due to delayed elimination of the toxin through the diminution of kidney substance thus allowing the toxin a longer interval to act on the susceptible cells of the body.

Reduction of kidney substance has a much more decided and pronounced effect on the L+ dose, a fact that is important in many respects. It is well known that the L+ dose is by far the most accurate and reliable index of the action and strength of diphtheria toxin, owing to the elimination of toxoid bodies. The decided reduction of the L+ dose in nephrectomized animals shows that diminution of the functional activity of the kidneys enhances the action of diphtheria toxin and lowers the efficacy of the antitoxins. The exact mechanism of this action cannot be easily proved. It may mean either the interference with the elimination of the toxin, the increased susceptibility of the tissue, or both. Moreover, nephrectomy may bring about the lowered resistance, in fact, must do so, at least in part by partial dissociation of the toxin-antitoxin combination. The L+ dose in the nephrectomized animals is so much reduced that no other explanation can be offered than that the decreased kidney function has increased the affinity for the toxin molecule so much that it is greater for the tissue cells than for the antitoxin molecule with liberation of free antitoxin. In other words, the toxin has a greater affinity for cells of low vitality than for antitoxin. This thus contributes further evidence to the theory advanced and supported by Roux and Vaillard,[4] Buchner,[5]

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[4] Ann. de l'Inst. Pasteur, 1893, 7, p. 65.

[5] München. Med. Wchschr., 1893, 40, p. 427.

Calmette,[6] Morgenroth,[7] and Arrhenius,[8] that the toxin-antitoxin combination is an unstable one and can be easily broken down with liberation of the toxin.

The importance of examining the urine of guinea-pigs used for standardization of diphtheria toxin and antitoxin for diminished or diseased kidney function is evident. In routine tests of guinea-pigs, it was found that approximately 2% of normal pigs and 17% of nephrectomized animals showed variable traces of albumin in the urine. In the course of these experiments a trace of albumin has played a very suggestive part in explaining the lowered resistance of 2 animals to the toxin. The wisdom of making titrations of toxins for standardization on a large series of control animals, as is done in some laboratories, is also apparent.

[6] Ann. de l'Inst. Pasteur, 1895, 9, p. 225.
[7] Berl. klin. Wchnschr., 1905, 50, p. 1550.
[8] Immunochemistry, 1907, p. 12.